

Studies on the Antiviral and Cytotoxic Activity of Schiff Bases Derived from 1,2-Bis-(*o*- and *p*-aminophenoxy)ethane and Salicylaldehyde

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A new synthesized derivative of substituted salicylaldehyde Schiff bases of aminophenoxy ethane were selected as new biological agents in the present study. The results indicate that none of the tested compounds was found to be an antiviral agent for DNA (bovine herpesvirus 1; BHV-1) and RNA (parainfluenza-3 viruses; PI-3) viruses used in this study but they possess highly cytotoxic effects.

Key Words: Schiff base, Cytotoxicity, Salicylaldehyde, Aminophenoxy, Antiviral activity.

INTRODUCTION

Many Schiff base complexes with metals have also provoked wide interest because they possess a diverse spectrum of biological and pharmaceutical activities, such as antitumour¹, antioxidative², antiviral³, antimicrobial⁴, antineoplastic⁵ activities and so on.

It is well known that salicylaldehyde Schiff base and thiosemicarbazone derivative of salicylaldehyde with other functional groups exhibit antiviral⁶ and antitumor activity^{7,8} and, moreover, aminophenoxy butyric acid derivatives are steroid-5 α -reductase inhibitors⁹. However, it appears that there is no report on the antiviral properties of Schiff bases derived from salicylaldehyde and aminophenoxy ethane in comparison with the information available for analogous SB. In view of these facts, newly synthesized substituted salicylaldehyde Schiff bases of aminophenoxy ethane were tested in the present study.

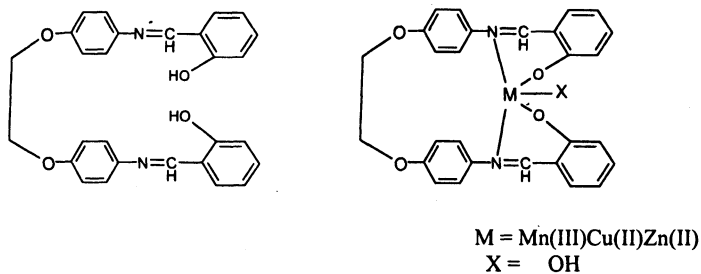
In this paper, Mn(II), Co(II), Cu(II), Zn(II), Ni(II) complexes of Schiff bases derived from condensation of 1,2-bis-(*o*- and *p*-aminophenoxy)ethane with salicylaldehyde (Figs. 1a and b) were screened for antiviral activity against Colorado strain of bovine herpesvirus-1 (BHV-1) and SF-4 strain of parainfluenza-3 virus (PI-3). In addition, the derivatives were tested for cytotoxicity effects on Madin-Darby bovine kidney (MDBK) and African green monkey kidney cells (VERO). Bovine herpesvirus-1 is the causative agent of infectious bovine rhinotracheitis/infectious pustular vulvo-vaginitis (IBR/IPV) disease of cattle and a member of family Herpesviridae containing a double-stranded DNA. Parainfluenza-3 virus, a member of the family Paramyxoviridae, genus paramyxovirus, is an enveloped

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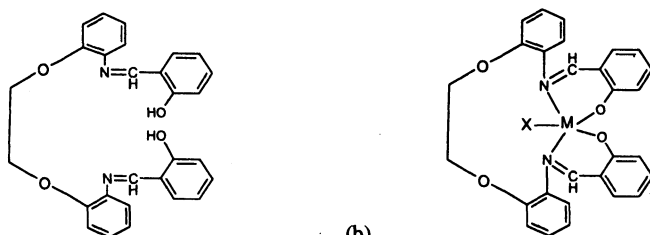
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virus containing a single stranded RNA molecule. This virus causes respiratory disease in cattle.

The synthesis and chemical properties of test compounds have been reported elsewhere^{10, 11}.



(a)



(b)

Fig. 1. (a) Structure of (N,N'-bis-(salicylidene)-1-2-bis(*p*-aminophenoxy)ethane (L_1), (b) (N,N'-Bis-(salicylidene)-1-2-bis(*o*-aminophenoxy)ethane (L_2) and suggested structure of the tetrahedral and square-planar complexes of the ligands.

EXPERIMENTAL

(N,N'-Bis-(salicylidene)-1-2-bis(*p*-aminophenoxy)ethane (L_1) and (N,N'-bis-(salicylidene)-1-2-bis(*o*-aminophenoxy)ethane (L_2) and their metal complexes were used in this study. Stock solutions of all compounds were prepared as 0.1 M solutions in dimethylsulfoxide (DMSO)-dimethylformamide (DMF) (1 : 1).

Cytotoxicity Assay: MDBK cells (2×10^5 cell/mL) on 24-well tissue culture plates were grown in cell growth media (Dulbecco's minimal essential medium supplemented with 10% fetal bovine serum and 100 IU/mL penicillin-100 μ g/mL streptomycin) containing the compounds dissolved in serial 4-fold dilutions ranging from 900 up to 1 μ M concentrations. The final concentration of DMSO-DMF in culture medium was always less than 0.5%, which does not induce cytotoxicity. In this study, 200 μ L of cell growth medium was added to the control cells. Each compound was tested in quadruplicate. After 96 h incubation, morphological changes in cells and cell increase were observed microscopically and with vital stain procedure by using trypan blue^{12, 13}. Cytotoxicity assay was performed also with VERO as described above.

Antiviral Activity: For the determination of virus inhibition effects of the compounds, initially, cells were grown as a monolayer on 24-well tissue culture plates. Then, cell growth media were removed and 100 μ L of virus inoculum in DMEM was added to each well at a multiplicity of infection of 0.05. After virus

adsorption for 45 min, 400 μL of test compounds diluted ranging from 400 to 5 μL concentrations into initially serial 4-fold dilutions with cell growth media were added to wells. Then, again, twofold dilutions were made in the same medium for compound's toxicity. Each concentration of compounds was tested in duplicate. Each experiment had cell control (no virus and no compound) and virus control (no compound). After 32 h post inoculation, the culture supernatants were collected for quantization of virus. Virus titres in supernatant were investigated with calculation of tissue culture infectious dose 50 (TCID₅₀). The assay was performed according to Spearman-Kärber method¹⁴.

RESULTS AND DISCUSSION

Test results of *in-vitro* screening are summarized in Table-1. Cytotoxicity is based on abnormal morphological changes observed in the cell monolayer. According to our results all compounds can affect the cells. The new derivatives were inhibitory to the growth of MDBK and VERO cells at concentrations ranging between 10-360 μM . For VERO cells, the cytotoxicity of compounds L₁, Cu-L₁, Zn-L₁, Mn-L₁ and L₂, Cu-L₂, Ni-L₂, Mn-L₂ are 32, 16, 78, 40, 312, 20, 80 and 312 μM respectively. For MDBK cells, the cytotoxicity of compounds L₁, Cu-L₁, Zn-L₁, Mn-L₁ and L₂, Cu-L₂, Ni-L₂, Mn-L₂ are 40, 12, 72, 48, 360, 16, 72 and 280 μM respectively. Therefore, it is clear that the cytotoxicity of L₁ and its complexes is greater than that of L₂ and its complexes.

TABLE-1
THE CYTOTOXIC EFFECTS OF L₁, L₂ AND METAL
COMPLEXES ON THE VERO AND MDBK CELLS

| Compounds | FW (g/mol) | Cytotoxicity (μM) in VERO cells | Cytotoxicity (μM) in MDBK cells |
|-------------------|------------|--|--|
| L ₁ | 452.00 | 32 | 40 |
| Cu-L ₁ | 513.90 | 16 | 12 |
| Zn-L ₁ | 511.40 | 78 | 72 |
| Mn-L ₁ | 521.90 | 40 | 48 |
| L ₂ | 452.00 | 312 | 360 |
| Cu-L ₂ | 513.50 | 20 | 16 |
| Ni-L ₂ | 508.71 | 80 | 72 |
| Mn-L ₂ | 521.90 | 312 | 280 |

In this study, virus inoculations were performed with TCID₅₀: $\log 10^{6.75}/\text{mL}$ for BHV-1 and TCID₅₀: $\log 10^{5.25}/\text{mL}$ for PI-3. In results of antiviral activity assay, cytopathic effects in cells added to non-cytotoxic dose of compounds and virus control cells were determined on same day. Moreover, CPE was increased in cells added to cytotoxic doses of compounds, in comparison with virus control cells. In the result of virus titration assays, it was shown that non-cytotoxic dose of compounds was not preventing production of BHV-1 and PI-3. Virus titration assay into cells added to cytotoxic dose of compounds was not performed. The data reported above clearly indicate that none of the tested compounds were found to be as an antiviral agent for BHV-1 and PI-3.

Wang *et al.*⁶, in order to have a better understanding of the correlation between the biological activities and the physicochemical properties of hydroxyamino-guanidine Schiff base derivatives, the molecular modifications cover a wide range of properties such as the lipophilicity, the size of the ring, the presence or absence of an *o*-OH group, the presence of an electron-donating group or electron-withdrawing groups on the ring and the orientation of substituent on the ring.

Our results confirm the orientation of substituent on the ring, *p*- or *o*- position. The linearity of the *para* position can cause high toxicity.

The cytotoxic effects of compounds Cu-L₁ and Cu-L₂ were distinctly superior to other L₁ and L₂ compounds. An explanation of the biological behaviour of complex Cu(L)₂ has been made by consideration of square-planar geometry⁷. The structures of Cu(II) compounds were square-planar. It has been reported that the structure and conformation of the ligand has influence on the redox potential of central atom in coordination compounds¹⁵. The changes (coordination sphere) of metal ions are connected with the change of diverse biological function of compound. The knowledge of these laws helps us to synthesize more active complexes or to understand the biological properties of natural biocoordinative compounds¹⁵. Our results confirm these explanations; the activity is affected strongly by the nature of the metal ions. The activities of the metal complexes are higher than that of the ligand.

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